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CASE REPORT

Misleading Free Thyroxine (FT4): A Case Report

Nurul Hidayah Hashim[1], Intan Nureslyna Samsudin[2], Ana Dalila Masiman[1], Subashini C. Thambiah[2]

[1] Department of Pathology, Hospital Melaka, 75400, Melaka, Malaysia
[2] Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400, Selangor, Malaysia

ABSTRACT

Interpretation of thyroid function test (TFT) is often straightforward but in certain scenarios, discordance between the clinical impression and the laboratory results exists. A 50-year-old woman with a ten years history of hypothyroidism on levothyroxine presented with a recent notable change in TFT [elevated free thyroxine (FT4) and thyroid-stimulating hormone (TSH)], in an otherwise clinically euthyroid and previously stable TFT, leading to levothyroxine being withheld. This case report highlights the possibility of assay interference as a cause of discordant TFT. It also draws the importance of close collaboration between clinicians and the laboratory to avoid unnecessary investigations and inappropriate management of such a case.

Keywords: Hypothyroidism, Immunoassay, Thyroxine, Thyroid function test (TFT)

Corresponding Author:
Intan Nureslyna Samsudin, MPath
Email: intanlyna@upm.edu.my
Tel: +603-97692374

INTRODUCTION

Thyroid stimulating hormone (TSH) and free thyroxine (FT4) tests are routinely requested for evaluation of thyroid function. Often, these thyroid function tests (TFTs) are easily interpreted in confirming the clinical diagnosis of hypothyroidism or hyperthyroidism. Occasionally, clinical and biochemical thyroid function mismatch, termed discordant TFT occurs, the pattern of which may point towards its possible causes, such as analytical interference in FT4 assay, as illustrated in this case. Failure to recognise such cause can lead to unnecessary investigations and inappropriate management (1).

CASE REPORT

A 50-year-old woman with known hypothyroidism on levothyroxine 50 micrograms daily for the past ten years presented with raised FT4 (27.5 pmol/L) and TSH (33.1 mIU/L) during a routine follow up (June 2016) at a primary-care centre (Table I). She was clinically euthyroid and her TFT results six months prior were normal. Three months later, a similar TFT pattern was obtained, resulting in the patient's levothyroxine being discontinued. Subsequently, the patient developed hypothyroidism symptoms mainly weight gain (6 kg within a month) and fatigue which corresponded to a TSH level of >150 mIU/L (November 2016) (Table I).

Table I: TFT results during follow up at the primary-care physician

	Modular E170 (Roche Diagnostics)		ADVIA Centaur XPT (Siemens Healthcare)	
Date/ Tests	FT4 (12.0 - 22.0 pmol/L)	TSH (0.27-4.20 mIU/L)	FT4 (11.5-22.7 pmol/L)	TSH (0.55-4.78 mIU/L)
December 2015	18.13	1.09	-	-
June 2016	-	-	27.5	33.10
September 2016	-	-	33.1	47.52
November 2016	-	-	17.0	> 150

She was restarted on levothyroxine 50 micrograms daily following a referral to the endocrinologist of Hospital Melaka. At this time, a history of occasional noncompliance was also elicited and she was advised to improve. Two months later (January 2017) (Table II) her FT4 remained elevated (27.6 pmol/L) and her TSH had normalised (64.05 mIU/L) while she was clinically euthyroid. Levothyroxine was decreased from once daily for 7 days to 5 days per week. All other biochemical investigations (renal profile, fasting lipid profile, liver function test, and full blood count) were normal. Her clinical and biochemical status remained status quo two months later (April 2017).

At this point of time, analytical interference in the TFT assays was considered as a possible cause of the discordant TFT. Biochemical tests from the health clinic at which patient was on follow-up were sent to Pathology Laboratory in Hospital Melaka. It was realised that the laboratory had changed its platform for TFT assays from Modular E170 (Roche Diagnostics) to ADVIA Centaur XPT (Siemens Healthcare) in the first quarter of

Table II: TFT results during follow up at Endocrine Clinic Hospital Melaka

Date/ Tests	ADVIA Centaur XPT (Siemens Healthcare) (Hospital Melaka)		Cobas e601 (Roche Diagnostics) (HKL)		UniCel Dxl 800 (Beckman Coulter, Inc) (HPJ)	
	FT4 (11.5-22.7 pmol/L)	TSH (0.55-4.78 mIU/L)	FT4 (12.0-22.0 pmol/L)	TSH (0.27-4.20 mIU/L)	FT4 (11.5-22.7 pmol/L)	TSH (0.55-4.78 mIU/L)
January 2017	27.6	64.05	-	-	-	-
April 2017	29.6	54.68	11.5	63.09	8.6	52.53
May 2017	25.3	85.50	-	-	-	-
July 2017	39.3	0.11	26.4	0.17	-	-
September 2017	27.7	0.32	18.8	0.40	-	-
December 2017	32.3	0.31	22.5	0.40	-	-
April 2018	34.1	0.24	23.4	0.25	-	-

2016. As part of the investigation, the patient's blood samples were sent to two different centres [Hospital Putrajaya (HPJ) and Hospital Kuala Lumpur (HKL)], which used UniCel Dxl 800 (Beckman Coulter) and Cobas e601 (Roche Diagnostics) platforms, respectively. In contrast to the results obtained on ADVIA Centaur XPT (Siemens Healthcare), the TFT results from both the centres showed low FT4 with raised TSH (Table II) consistent with the patient's initial diagnosis. Her levothyroxine dose was increased to 100 micrograms daily. A diagnosis of hypothyroidism on treatment with discordant TFT secondary to FT4 immunoassay interference was made. The patient continued to be on follow-up at the endocrine clinic whereby her levothyroxine dosages were adjusted clinically, guided by her Cobas e601 (Roche Diagnostics) TFT results. As she remained asymptomatic, further investigations such as imaging and other specific laboratory tests to rule out other causes of discordant TFT were not performed.

DISCUSSION

The causes of hyperthyroxinaemia with raised TSH, as seen in this patient include a) assay interference to FT4 or TSH assays, b) non-thyroidal illness (NTI), c) TSH secreting pituitary adenoma (TSHoma), d) resistance to thyroid hormone (RTH) and e) medications such as amiodarone and thyroxine replacement therapy (including poor compliance) (2). Having no history of acute or chronic illnesses as supported by normal routine blood investigations, and prescription of other medications, exclude NTI and medication as causes of the discordant TFT. TSHoma which would result in hyperthyroidism, was unlikely given her longstanding history of hypothyroidism and the drastic normalisation of TSH following adequate levothyroxine dosages. RTH was also unlikely as affected patient would otherwise be clinically euthyroid. Hence investigations such as pituitary MRI scanning and TRH test were not performed.

Discordant TFT (increased TSH/increased FT4) may be caused by continuous disequilibrium between FT4 and TSH (2). In this patient, the occasional noncompliance was, reflected by her elevated TSH levels from June-September 2016, which revealed her true clinical status despite being asymptomatic. Furthermore, it was misled by increased FT4 levels. Discontinuation of levothyroxine eventually unmasked her true clinical state of hypothyroidism in November 2016, which was biochemically evident by a significantly elevated TSH. Although the FT4 had decreased to within the reference interval, it was actually falsely high. A sudden alteration in the pattern of the patient's TFT results following the change of the laboratory's analyser should have hinted the possibility of assay interference, given the previously stable TFTs.

FT4 is often measured using a competitive immunoassay method (1). Known FT4 immunoassay interference includes biotin, heterophile antibodies, human anti-animal antibodies (HAAA) and thyroid hormone autoantibodies (THAAb) (1). These interferents may alter the FT4 measurements depending on the immunoassay format and types of reagent antibodies, immobilising and detection systems used (1). In this case, the interference in the ADVIA Centaur XPT (Siemens Healthcare) FT4 assay had resulted in falsely higher values whilst the other FT4 assays were unaffected. Although all three are competitive immunoassays, they differ in several aspects (Table III). The ADVIA Centaur XPT (Siemens Healthcare) FT4 is a one-step assay, in which the endogenous FT4 in the patient's serum competes with the assay's labelled T4 analogue for binding to a limited amount of biotinylated polyclonal rabbit anti-T4 antibody immobilised to a solid surface. Any unbound fraction is removed during a washing step and the

Table III: Principles of FT4 immunoassays

Characteristics	ADVIA Centaur XPT (Siemens Healthcare)	Cobas e601 (Roche Diagnostics)	UniCel Dxl 800 (Beckman Coulter, Inc)
Type	One-step competitive	One-step competitive	Two-step competitive
Steps	One incubation and one wash	Two incubations, one-wash	Two incubations, two-wash
Antibody	Polyclonal rabbit anti-T4 antibody, coupled to biotin	Polyclonal sheep anti–T4 antibody, labelled with ruthenium complex	Monoclonal mouse anti-T4 antibody, coupled to biotin
T4 analogue	T4 labelled with acridinium ester	T4 coupled with biotin	T4 labelled
Solid phase	avidin-coated paramagnetic microparticles	streptavidin-coated paramagnetic microparticles	streptavidin-coated paramagnetic microparticles

signal generated from the bound labelled T4 analogue is measured. The Cobas e601 (Roche Diagnostics) FT4 assay is also a one-step immunoassay but is performed in two sequential incubations with one washing step at the end. In contrast, the UniCel Dxl 800 (Beckman Coulter) FT4 is a two-step assay with washing steps in between and at the end.

The three immunoassay systems exploit the biotin-streptavidin interaction leaving them potentially susceptible to biotin interference with increasingly reported cases over recent years (3,4). The magnitude of interference, however, is dependent on the extent of excess biotin in the sample and the interference threshold of the assay (3,4). The excess biotin will bind to the streptavidin-solid surface and prevents immobilisation of biotinylated antibody leading to falsely increased result in competitive immunoassays. In this patient, biotin was an improbable cause for the interference as the patient had no history of biotin intake. In addition, ADVIA Centaur XPT (Siemens Healthcare) uses preformed biotin-avidin solid phase, making it insensitive to biotin interference, compared to non-preformed system (5).

Patient's samples may contain heterophilic or human anti-animal antibodies (HAAA) that are capable of binding to the animal antibodies used in the immunoassay system. By definition, HAAA are high-affinity antibodies that developed towards specific animal epitopes (e.g. mice and rabbits) while heterophilic antibodies are weak antibodies formed in response to poorly defined antigens (4). In patients with no history of receiving animal monoclonal immunoglobulin (MAb) injections such as in this patient, the term heterophilic antibody is preferred. However, this was unlikely in this patient as such interference more commonly affects non-competitive assay such as TSH, compared to competitive assay (4).

THAAb directed against T4 is a possible cause of FT4 assay interference in this patient. THAAb is present in <2% of the general population but in as high as 40% in those with autoimmune thyroid disease (AITD) (4). Between 80-100% of samples with THAAb are positive for anti-thyroid peroxidase or anti-thyroglobulin antibodies although these were never measured during this patient's 10-year history of hypothyroidism (1,4). THAAb tends to affect one-step assays as it forms a complex with the labelled T4 analogue preventing the complex from binding to the solid phase and hence leading to a falsely elevated FT4. Two-step assay, as utilised by UniCel Dxl 800 (Beckman Coulter), on the other hand, is considered less sensitive towards these autoantibodies since the THAAb are removed during the intermediate washing step, before the introduction of the labelled T4 analogue (4). Similarly, staggered incubation in one-step assay on Cobas e601 (Roche Diagnostics) limits the duration of contact between THAAb and the T4 analogue, rendering it less sensitive to this interferent (4), as shown in Table II. As AITD is one of the commonest cause of hypothyroidism, THAAb seems to be the most likely interferent in FT4 assay in this case.

A hyperthyroxinaemia with raised TSH in a patient with a longstanding history of hypothyroidism was highly suspicious of assay interference. As such, retesting with other immunoassay platforms was a good initial approach (1), as demonstrated in this case. Other investigations for suspected interference include a demonstration of nonlinearity with sample dilution, removal of interfering antibodies using a nonimmune serum or heterophilic blocking tubes, or precipitation with polyethylene glycol (2). These tests were, however, not performed due to non-availability and limited resources. Ideally, one should elicit the underlying cause of such assay interference and findings should be documented to avoid future mismanagement.

CONCLUSION

Immunoassay is prone to interference (4). Both the clinicians and laboratorians need to be mindful of such probability in the differential diagnosis of discordant TFT. In any case, not only correlation with clinical history is mandated, efforts should also be given in eliciting the underlying cause of assay interference.

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